REMARKS

Upon entry of this amendment, claims 201, 209, 212-214, 239, and 244-248 are pending in the application. Claims 201, 212, 244, and 245 are amended herein. New claims 246-248 are added. Support for the new claims and claim amendments is found in the original claims and throughout the instant application as-filed, *e.g.*, at least at page 73, lines 11-14; page 91 at lines 1-2; and at Example 1 of the specification. Support for the amendment to claim 201 can additionally be found at least at page 8, lines 30-31 and page 22, lines 13-21 of WO99/40783 to Peled ("Peled"), which is incorporated by reference at page 3, lines 27-28 of the instant specification as-filed. No new matter is added.

Supplemental Information Disclosure Statement

Applicants submit herewith a Supplemental Information Disclosure Statement for full consideration by the Examiner.

35 U.S.C. § 112, First Paragraph Rejections

Claims 201, 209, 212-214, 239, 244 and 245 are rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. *See*, Office Action at pages 3-7. Applicants traverse the rejection with respect to the pending claims as amended herein.

The Examiner, while acknowledging that the specification is enabling for:

a method of expanding an *ex-vivo* population of CD34+ hematopoietic stem cells (HSC) in culture medium, while at the same time inhibiting differentiation of the cells *ex-vivo* in a culture medium, the method comprising: (a) providing hematopoietic mononuclear cells that are not enriched prior to culturing, (b) culturing the mononuclear cells (MNC) *ex-vivo* for a period greater than 7 days in culture under conditions allowing for proliferation in the presence of FLT3, IL-6, TPO and SCF, and at the same time inhibiting differentiation in the presence of an effective amount of at least one copper chelator TEPA, which reduces intracellular copper concentration in said cells; thereby expanding the population of said hematopoietic stem cells while at the same time inhibiting the differentiation of said hematopoietic stem cells *ex-vivo* in culture for a period of greater than 7 days;

has alleged that the specification does not provide enablement for culturing mononuclear cells under conditions comprising any copper chelator at any concentration, in an undefined medium lacking cytokines, as broadly claimed. *See*, Office Action at page 3. Applicants disagree.

Regarding cytokines and conditions for proliferation of the hematopoietic stem cells, the Examiner states that it is apparent that one skilled in the art would have to make a new discovery to determine the right combination of early and late cytokines with the appropriate concentration of the copper chelator for expanding an *ex-vivo* population of HSC. *See*, Office Action at page 5. Applicants submit that not only the instant specification, but also Murray *et al.* 1999, Exp Hematol, 27:1019-1028 ("Murray"; already of record) and Peters *et al.* 2002, Br J Haematol, 119:792-802 ("Peters"; already of record) teach that no single "right combination" of cytokines for *ex-vivo* proliferation of hematopoietic stem cells exists. Rather, a variety of conditions are suitable for use with the methods as claimed.

The Examiner's attention is directed to Example 1 of the instant specification, which discloses that cytokines FLt-3, IL-6, TPO and SCF were used for *ex-vivo* expansion of hematopoietic stem cells, wherein SCF was occasionally replaced with IL-3. Moreover, contrary to the Examiner's contention, Murray does not show that cytokine combinations have "profoundly different effects". Rather, Murray shows that a large number of cytokine combinations produce similar results in % viability of the cells (*see*, Murray at FIG. 2A) and in fold-increase of CD34+ Thy+ cells at 90 hours (*see*, Murray at FIG. 5).

Furthermore, rather than showing that there is a single "right combination" of cytokines for *ex-vivo* expansion of hematopoietic stem cells for a period greater than 7 days, Peters states that "With most conditions, the maximum expansion was obtained at 11-15d...". *See*, Peters at page 794, right column, Results. While Peters chose only a single condition for stem cell expansion beyond 50 days, clearly other combinations of conditions provided expansion for greater than 7 days culture. Further, it is curious to note that using other conditions for HSC expansion reported effective in the literature (such as those of Piacibello *et al.*, 1997 Blood, 89(8):2644-2653 ("Piacibello"; already of record)), Peters failed to achieve HSC expansion (*see*, Peters at page 794, right column, Results), raising suspicion

regarding the universality of the conclusions reached in Peters.

Thus, Applicants submit that neither Murray nor Peters, nor the state of the art at the time of filing, indicate the existence of a single "right combination" of cytokines for *ex-vivo* expansion of hematopoietic stem cells. Rather, one of ordinary skill in the art may need to perform routine optimization of *ex-vivo* culture conditions, as taught at page 88, lines 5-14 of the instant specification:

> "Preferably, culturing the hematopoietic mononuclear cells is performed in a presence of an effective amount of a cytokine, preferably, an early acting cytokine or a combination of such cytokines, e.g., thrombopoietin (TPO), interleukin-6 (IL-6), an FLT-3 ligand and stem cell factor (SCF). This assay can be used, by one ordinarily skilled in the art, to determine, for example, which of the antagonists, inhibitors or copper chelators and chelates listed above is most efficient for the purpose of implementing the various methods and preparations of the present invention described hereinabove. The assay can be further used to determine most effective concentrations and exposure time for achieving optimal results with hematopoietic mononuclear cells of different origins."

Regarding linear polyamine chelators, the Examiner alleges that no guidance is provided with respect to polyamine copper chelators other than TEPA, and that "neither specification nor prior art establishes any nexus between expansion of CD34+ cells in MNC to presence of any copper chelator other then that is exemplified in the instant application...". *See*, Office Action at page 5. Applicants disagree.

The above-mentioned notwithstanding, and in order to expedite prosecution in this case, claim 201 is amended herein to include the limitations of culturing the mononuclear cells in a combination of cytokines selected from the group consisting of TPO, IL-6, FLT-3 ligand, SCF and IL-3, and an amount of TEPA effective in reducing intracellular copper concentration. Support for this amendment is found throughout the instant specification, *e.g.*, at least at Example 1 of the specification. Thus, amended claim 201, and claims dependent therefrom, clearly define the culture conditions for proliferation of CD34+ cells and reduction of the copper concentration resulting in inhibition of differentiation of CD34+ cells for a period of at least 14 days, such that one of ordinary skill in the art would be able to practice the method as claimed without extensive or undue experimentation.

Thus, Applicants submit that the pending claims, as amended herein, are fully enabled by the instant specification such that one of ordinary skill in the art could make and use the invention without undue experimentation. Reconsideration and withdrawal of the present rejection are respectfully requested.

35 U.S.C. § 103 Rejection

Claims 201, 209, 212-214, 239, 244 and 245 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Fietz *et al.*, 1999 Bone Marrow Transplant, 23(11):1109-1115 ("Fietz") or Wang *et al.*, 2002 Sheng Wu Gong Cheng Xue Bao 18(3):343-347 ("Wang") and WO99/40783 to Peled ("Peled"). *See*, Office Action at pages 8-10. Applicants traverse the rejection with respect to the pending claims as amended herein.

In order to expedite prosecution in this case, claim 201 is amended herein to include the limitations of removing one half of the culture volume and replacing it with fresh medium and cytokines weekly, and *ex-vivo* culture of the hematopoietic mononuclear cells for a period of at least 14 days. Support for these limitations can be found throughout the instant specification, *e.g.*, at least at Example 1 of the specification. Support for the amendment to claim 201 can additionally be found at least at page 8, lines 30-31 and page 22, lines 13-21 of WO99/40783 to Peled ("Peled"), which is incorporated by reference at page 3, lines 27-28 of the instant specification as-filed.

In rejecting the claims over Fietz or Wang and Peled, the Examiner states that "for obviousness under 35 U.S.C. § 103, all that is required is a reasonable expectation of success". The Examiner further states that the Applicants have been arguing against the references individually, where the rejection is based on combinations of references. *See*, Office Action at pages 8-9. Applicant disagrees.

The Examiner alleges that, in view of the teachings of Fietz and Peled, one of skill in the art would have been capable of applying, with a reasonable expectation of achieving predictable results, the known technique (expanding CD34+ cells with cytokines and a copper chelator) to the known methods of culturing unselected cells as in Fietz, based on the assertion that:

> "Fietz had already described a method of *ex-vivo* expansion of...(MNCs), in the presence of early and late acting cytokine for one to four week, while Peled described use of copper chelator such as TEPA that could facilitate expansion of CD34+ cells beyond one week by inhibiting differentiation of CD34+ cells."
> Office Action, page 10.

Applicants wish to point out that at the time of filing the instant specification, the *ex-vivo* expansion methods of Peled could not be applied to the teachings of Fietz, with a reasonable expectation of success by one of skill in the art. As noted by the Examiner, Fietz failed to observe any significant increase in total or CD34+ cells with greater than 7 days culture of the unselected mononuclear cells. Furthermore, Fietz reported that while selected CD34+ cells thrived following refeeding and reseeding, the "MNC fraction did not profit from refeeding and reseeding. Cell numbers decreased after refeeding and reseeding, as did the percentage of CD34+ cells" (see, Fietz at page 1111), and MNCs only proliferated in static culture conditions. This is incompatible with the culture method taught by Peled, which requires removing one half of the culture volume and replacing it with fresh medium and cytokines weekly. *See*, Peled at Example 1, Culture Procedures, page 22, lines 13-21, and at Example 3, page 27, lines 13-18. Thus, Fietz teaches away from the claimed invention.

Therefore, one of ordinary skill in the art, in possession of evidence against expanding hematopoietic stem cells from unselected mononuclear cells, (as taught by McNeice *et al.*, 2004 Cytotherapy, 6(4):311-317 and Briddell *et al.*, 1997 J Hematother, 6(2):145-150 (both references already of record)), would assume that the culture methods taught by Peled, requiring refeeding and reseeding, while suitable for expansion of purified CD34+ fractions, could not be applied to the teachings of Fietz with a reasonable expectation of success.

Wang does not cure the deficiencies of Fietz, inasmuch as Wang reports total cell proliferation of MNCs for 4 weeks only, with poor results (50 fold increase versus >30,000 fold increase in CD34+ selected culture), poor CD34+ expansion for only 7 days in the unselected MNC cultures (50 ± 33.2 fold), and clearly recommend against ex-vivo expansion of CD34+ cells from unselected MNCs for more than 7 days. According to Wang, the results showed that CD34+ selected cells culture could obtain more CFU-GM cells and CD34+ cells

during the whole period. Moreover, Wang is silent with regard to the refeeding and reseeding of cultures.

Thus, Applicants submit that the combination of Fietz or Wang and Peled fail to teach or suggest all the limitations of the claimed invention, and as such, do not constitute *prima facie* evidence for obviousness. Further, Applicants submit that one of ordinary skill in the art reading the combination of Fietz or Wang and Peled would have no reasonable expectation of success in reaching the claimed invention. Reconsideration and withdrawal are respectfully requested.

Double Patenting Rejections

Claims 201, 209, 212-214, 239 and 244-245 are rejected for obviousness-type double patenting as being unpatentable over claims 1-11 of U.S. Patent No. 7,169,605. Claims 201, 209, 212-214, 239 and 244-245 are provisionally rejected for obviousness-type double patenting as being unpatentable over claims 1-6, 8-17, 19-22, 123-131 of copending U.S. Patent Application No: 10/418,639 and claims 1, 2-11 and 23 of copending U.S. Patent Application No: 10/564,777. Applicants will consider filing a terminal disclaimer upon notice of allowable subject matter in this application.

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that the claims are in condition for allowance. Should any questions or issues arise concerning the application, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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Dated: February 5, 2009

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